Improved target weight optimization in *phenix.refine*

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Abstract

Restrained refinement of individual atomic coordinates and atomic displacement parameters combines experimental observations with prior knowledge. The two contributions need to be properly weighted with respect to each other in order to obtain the best results. This article describes a new target weight determination procedure in *phenix.refine* and presents the results of systematic tests on structures with lower resolution data.

Introduction

In *phenix.refine* (Afonine et al., 2005, Adams et al., 2010) the refinement of individual atomic coordinates or individual atomic displacement parameters (ADP, also known as B-factors) involves the minimization of a refinement target function that includes prior chemical or empirical knowledge. In the case of individual coordinate refinement this target function T is defined as:

$$T = w_{xc} \cdot w_{xc\_scale} \cdot T_{data} + w_c \cdot T_{geo\_restraints} \quad (1)$$

T_{data} is the target function quantifying the fit of experimental observations (X-ray and/or neutron data) and model-based predictions, using, for example, a least-squares or maximum-likelihood function. $T_{geo\_restraints}$ quantifies the fit of current model geometry (such as bonds, angles, dihedrals and nonbonded interactions) to tabulated "ideal" geometry, for example as inferred from high-resolution diffraction experiments. The three weight factors w_{xc}, w_{xc_scale} and w_c are redundant; equation (1) could be reformulated with only one weight factor. However, the formulation with three weight factors is helpful in practice. This is also true for the analogous formulation used in ADP refinement:

$$T = w_{xu} \cdot w_{xu\_scale} \cdot T_{data} + w_u \cdot T_{ADP\_restraints} \quad (2)$$

The weight factors w_c and w_u are usually one, but can be set to zero for unrestrained refinement. The weights w_{xc} and w_{xu} are determined automatically as described by Brünger *et al.* (1989) and Adams *et al.* (1997), using the ratio of the gradient norms after removing outliers:

$$w_{xc} = \sqrt{\frac{\langle \nabla T_{geo\_restraints}{}^2 \rangle}{\langle \nabla T_{data}{}^2 \rangle}} \quad (3)$$

$$w_{xu} = \sqrt{\frac{\langle \nabla T_{ADP\_restraints}{}^2 \rangle}{\langle \nabla T_{data}{}^2 \rangle}} \quad (4)$$

w_{xc_scale} and w_{xu_scale} are empirical scale factors, usually with values between 0.5 and 1.0.

An automatic weight determination procedure based on equations (3) and (4) has been used in *phenix.refine* from the beginning of its development. The procedure is usually reliable at typical macromolecular resolutions (around 1.5-2.5 Å) but sometimes problematic at significantly lower (> 3 Å) or higher (< 1.5 Å) resolutions. Typical problems are unexpectedly high R_{free} values, large gaps between R_{free} and R_{work}, unreasonably large geometry deviations from ideality, high Molprobity clash-scores, or large differences between ADPs of bonded atoms.

Brünger (1992) described a procedure that systematically searches for the weight leading to the lowest R_{free}. Until recently, the implementation in *phenix.refine* used an array of 10-20 values for w_{xc_scale} or w_{xu_scale}, with values distributed between 0.05 and 10. A full trial refinement was performed for each weight. In our experience, using R_{free} as the only guide for determining the optimal weight can sometimes discard results that are clearly more preferable if other quality measures are also taken into account. For example, R_{free} may oscillate only slightly while R_{work}, bond and angle deviations, or clash-scores change significantly. In this article we describe an enhanced weight search procedure that makes active use of an ensemble of quality measures.

Methods

In contrast to the previously used procedure, the new procedure in *phenix.refine* examines trial

weights on an absolute scale:

$$T = w_{trial} \cdot T_{data} + T_{restraints} \qquad (5)$$

Since *phenix.refine* uses normalized targets for data and restraints, the range of plausible values is predictable. For example, the amplitude-based ML target (Lunin & Skovoroda, 1995; Afonine *et al.*, 2005) typically yields values that fall in the range between 1 and 10 (depending on resolution, data quality and model quality). The weight optimization procedure is parameterized with a spectrum of trial weights that is sufficiently large to offset such variations in the scale of T_{data}.

The new procedure executes the following steps:

1. For each trial weight, perform 25 iterations of LBFGS minimization (Liu & Nocedal, 1989) and save R_{work}, R_{free}, R_{free} - R_{work}. For coordinate refinement, also save bond and angle RMSDs and the clash-score. For ADP refinement, also save the mean difference between B-factors of bonded atoms $<\Delta B_{ij}>$.
2. Select the subset of plausible results corresponding to R_{free} values in the range $[R_{free}^{min}, R_{free}^{min} + \Delta]$, where Δ is a resolution-dependent value in the range from 0 (high resolution) to 2% (low resolution) and R_{free}^{min} is the smallest R_{free} value obtained in step 1.
3. Reduce the subset further by applying selection criteria based on the R_{free} - R_{work} difference and bond and angle RMSDs (coordinate refinement) or $<\Delta B_{ij}>$ (ADP refinement).
4. In the case of coordinate refinement, reduce the subset further based on the clashscores (c). The first step is to the select results that satisfy the condition $\bar{c}/3 < c < 3\bar{c}$. For the second step recompute the mean $\bar{c}_{new}$ for the new subset and select results in the range from the minimum of the clashscores, c_{new}^{min}, to $c_{new}^{min} + w_{cs} * \bar{c}_{new}$. Currently the default value for w_{cs} is 0.1.
5. For the remaining subset select the result that corresponds to the lowest R_{free}.

The choice of Δ values in step 2 is based on the evaluation of a large number of refinements. We selected a number of data/model pairs covering a range of resolutions. For each pair we ran multiple refinements with identical parameters, except for the random seed used in the target weight determination and the simulated annealing module. In another series of tests, we applied modest random shifts to coordinates and *B*-factors before refinement. An ensemble of similar solutions is obtained for each data/model pair. Identical solutions cannot be expected (for example, see Terwilliger *et al.*, 2007) because the refinement target function is very complex and populated with many local minima; therefore the starting point is important. In addition, the structural deviations can be a consequence of static or dynamic disorder that is difficult to model. The Δ values reflect typical R_{free} fluctuations we observed in our refinement results, ranging from small fractions of a percent for high-resolution refinements and approaching 2 percentage points in a few low-resolution cases.

The optimal value for $<\Delta B_{ij}>$ in step 3 is not clearly defined, as discussed in Afonine *et al.* (2010a). Our current working estimate is $0.1<B>$, where $<B>$ is the average *B*-factor.

The weight optimization procedure is easy to parallelize since the refinements with different trial weights are independent. Starting with PHENIX version dev-810, the `refinement.main.nproc` parameter is available to specify the number of CPUs the weight optimization procedure may use in parallel. To give one example, the command

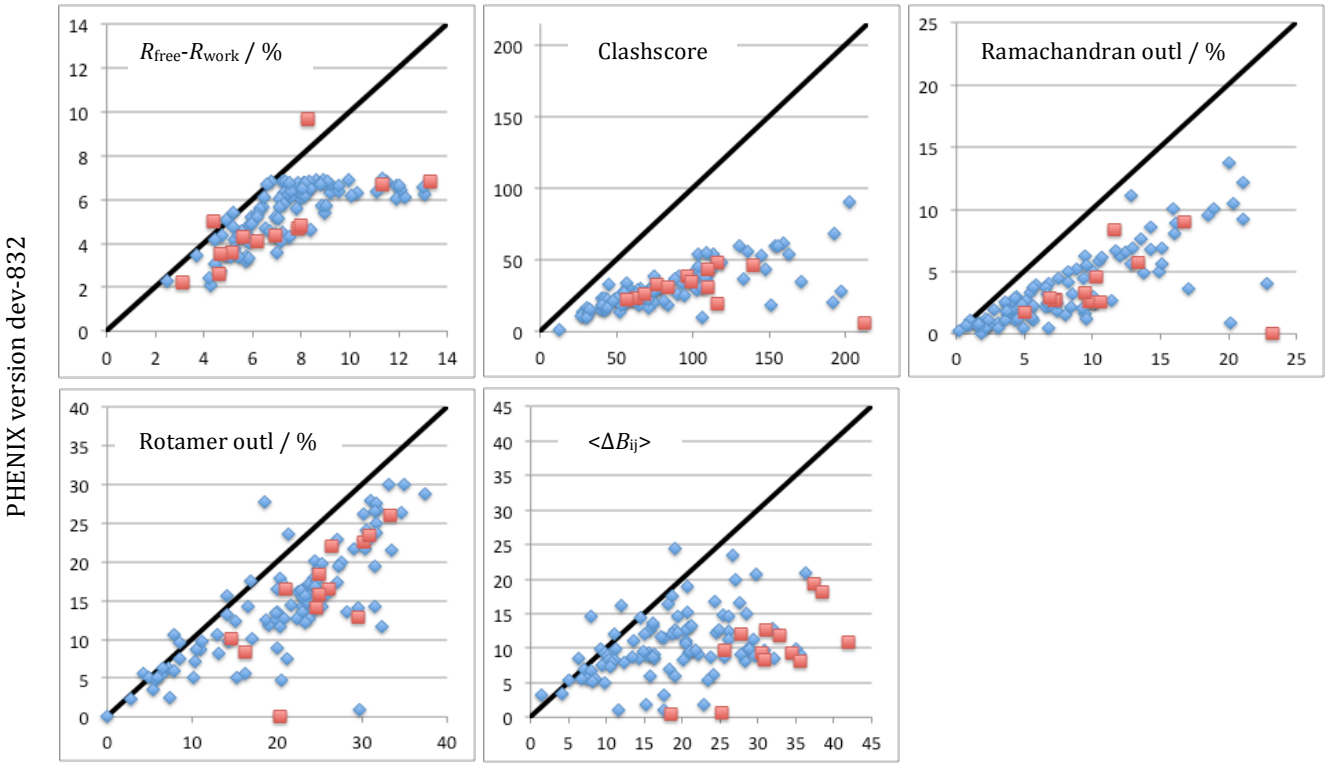
```
phenix.refine 1av1.pdb 1av1.mtz \
  optimize_xyz_weight=True \
  optimize_adp_weight=True nproc=16
```

finishes in approximately 430 seconds on a 48-core 2.2GHz AMD Opteron system. With `nproc=1` the refinement requires more than 2000 seconds on the same machine.

Results and discussion

To evaluate the new procedure we selected a set of low-resolution structures from the PDB (Bernstein *et al.*, 1977; Berman *et al.*, 2000) using on the following criteria:

- data high resolution limit between 3.5 and 4.5 Å,
- data completeness (overall and 6 Å – inf) better than 85%,
- data collected from untwinned crystals,



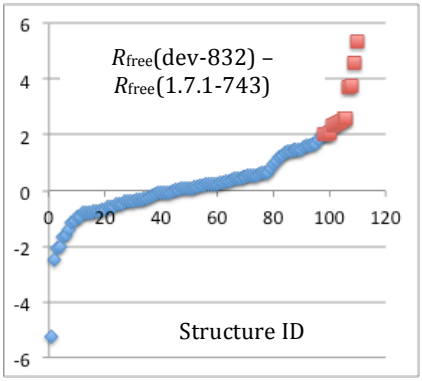
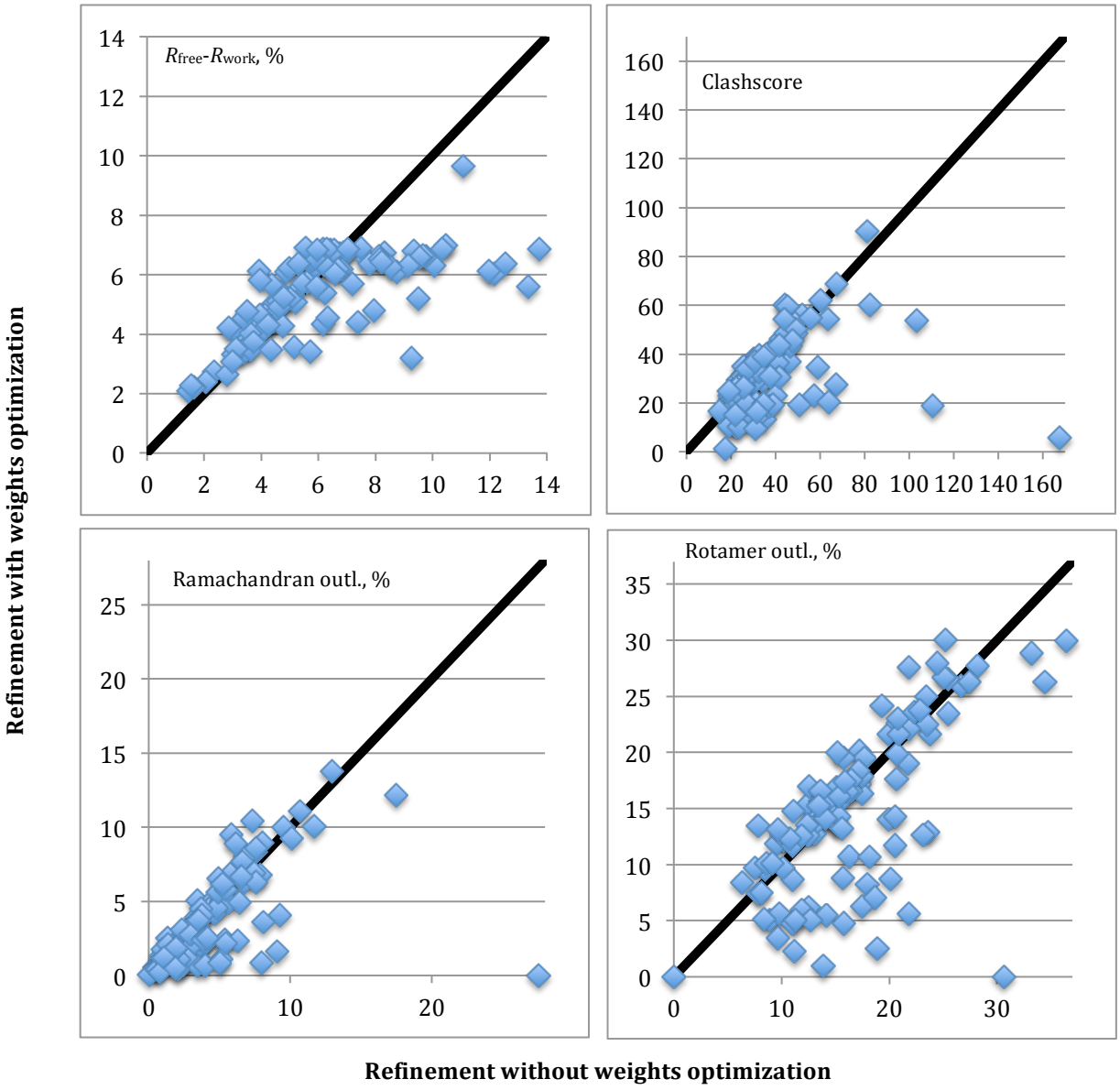


Figure 1. Comparison of refinement results between two PHENIX versions 1.7.1-743 and dev-832 (see text for details). Red squares highlight cases where the difference in R_{free} was larger than 2 percentage points (see the last plot where the results are ordered by R_{free} difference between the runs).

- data extractable from the PDB archive using *phenix.cif_as_mtz* (Afonine *et al.*, 2010b),
- models consisting only of common protein residues, ligands, heavy atoms and water,
- non-zero occupancy for all atoms,
- R_{free} flags available and a minimal gap between R_{free} and R_{work} of more than 2 percentage points.

We found 108 matching structures. Each structure was refined with 5 macro-cycles of restrained refinement of individual coordinates and ADPs (Afonine *et al.*, 2005). Most selected structures contain NCS-related molecules. NCS-related groups were determined automatically by *phenix.refine* and restrained in Cartesian space (atom-pair-wise harmonic restraints to the group average). Refinements were performed using the old weight optimization procedure as available in PHENIX (Adams *et al.*, 2002; Adams *et al.*, 2010) version 1.7.1-743 and the new procedure as included in a current development version (dev-832). The detailed results are presented in Figures 1 and 2.

Figure 1 compares refinement results (R_{free} - R_{work} difference, clash-score, <ΔB_{ij}>, percent of rotamer outliers and Ramachandran plot outliers) between *phenix.refine* runs using PHENIX versions 1.7.1-743 and dev-832. The results show clearly that the new procedure provides much improved geometry statistics and lower <ΔB_{ij}> values in most cases. The reduction of Ramachandran and rotamer outliers is especially noteworthy, since these quality measures are not directly used in the target weight optimization. The R_{free} comparison shows that the deviations are mostly within the Δ parameter range (2 percentage points), as



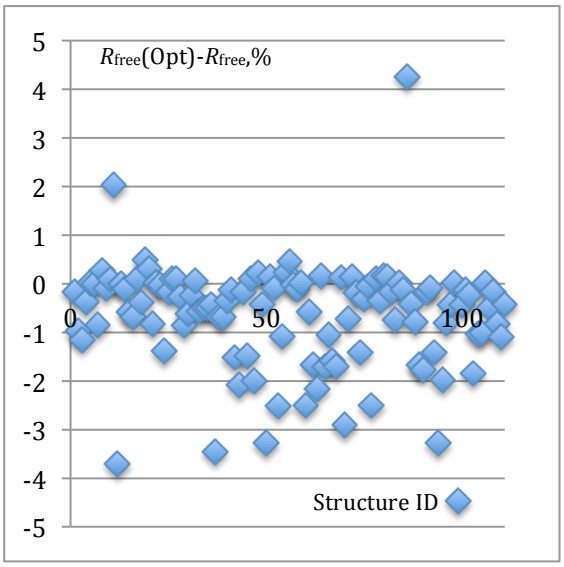


Figure 2. Results of refinement with and without weights optimization using PHENIX version dev-832.

expected. The few outliers with differences larger than 2 percentage points (red squares on Fig. 1) may be due to non-optimal NCS group selections that require further analysis, or $<\Delta B_{ij}>$ values that were forced to obey the requested limit. The large number of Ramachandran plot outliers may also indicate problems with the starting models that are beyond the anticipated convergence radius of these refinement procedures. Examining these cases in detail may lead to further improvements.

Figure 2 shows a comparison of statistics similar to Figure 1, after refinement with and without weight optimization using the current PHENIX development version (dev-832 or later).

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